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# Antibiotic use during pregnancy and neurodevelopmental outcomes of offspring in early childhood

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Thesis

# ANTIBIOTIC USE DURING PREGNANCY AND NEURODEVELOPMENTAL OUTCOMES OF OFFSPRING IN EARLY CHILDHOOD

by

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ABSTRACT

There is limited research on the effects of antibiotic use during pregnancy on neurodevelopmental outcomes of offspring in early childhood. The aim of this study was to investigate associations between antibiotic use during early pregnancy and neurodevelopmental outcomes, both behavioral and cognitive, in the offspring during early childhood. This thesis examined a longitudinal study of 570 mother-child pairs where prenatal exposures and at least one neurodevelopment outcome assessment were recorded. An interview was conducted with mothers on average one year after delivery to collect information on prenatal exposures. Neurodevelopmental outcomes were assessed between the ages 5–11 years using the cognitive-based outcomes of Peabody Picture Vocabulary Test (PPVT-III) and the Beery-Buktenica Test of Visual Motor Integration-Fifth Edition (VMI-5) and behavioral-based outcomes of the Child Behavior Checklist (CBCL) and Teacher Report Form (TRF). Adjusted mean differences (adjMD) in outcome measures were calculated between mothers reporting antibiotics use and mothers reporting treated infections. Antibiotic use during pregnancy was not significantly associated with the two cognitive measures but was associated with increased total behavioral problems reported by mothers (adjMD: 2.60; CI: 0.50, 4.69) and teachers (adjMD 2.60; 95% CI 0.44, 4.76). Overall, antibiotics use during pregnancy was not associated with differences in childhood cognition but may be associated with

greater behavior problems.

*Keywords*: antibiotics, pregnancy, neurodevelopment, cognition, behavior

## Table of Contents

**List of Tables**

## List of Abbreviations

ASD……...……Autism Spectrum Disorders

BMI……Body Mass Index

CBCL……Child Behavior Checklist

CI…….…..……..Confidence Interval

HFM……...……..Hemifacial Microsomia

HIPAA……Health Insurance and Accountability Portability Act

HR……..……...……....Hazard Ratio

IRB……..……...….Institutional Review Board

MC……..……...….Maternal Chorioamnionitis

MD……..……...….Mean Difference

NOS……Not Otherwise Specified

PPVT-III……Peabody Picture Vocabulary Test-Third Edition

SD……Standard Deviation

TRF……Teacher Report Form

UGI……Urogenital Infection

URI…….Upper Respiratory Infection

VMI-5…….Beery-Buktenica Development Test of Visual Motor Integration-Fifth Edition

# Antibiotic Use During Pregnancy and Neurodevelopmental Outcomes of Offspring in Early Childhood

Over the course of pregnancy, there are dramatic changes in the composition of the maternal gut microbiota which influences the long-term health of both mother and child (Cenit, Sanz, & Codoñer-Franch, 2017). The gut microbiota is considered a regulator along the gut-brain axis and maternal gut microbiota may play a key role in neurodevelopment and neurodegenerative disorders of offspring (Cenit, Sanz, & Codoñer-Franch, 2017). Antibiotic use during pregnancy profoundly affects maternal microbiota which shapes the microbial colonization of the infant gastrointestinal tract of the fetus (Gonzalez-Perez, et al., 2016). Therefore, with side effects known to disrupt the microbiome, antibiotics may in turn have effects on this gut-brain axis and consequently, also affect neurodevelopment.

Antibiotics are beneficial and relatively safe during pregnancy with approximately 8% to 20% of women being prescribed antibiotics during pregnancy (Amann, et al., 2006; Czeizel, et al., 1998; Headley, et al., 2004). Antibiotic treatment accounts for 80% of prescribed medications during this period (Kuperman & Koren, 2016; Landers, et al., 1983; Mitchell, et al., 2011). Despite the frequency of antibiotic use during pregnancy and likely effects of antibiotics on the gut-brain axis of both the mother and fetus, there has been limited research on outcomes related to prenatal exposure to antibiotics (Czeizel, et al., 1998; Cenit, Sanz, & Codoñer-Franch, 2017).

One study of antibiotic use during pregnancy has suggested an association with increased incidence of autism spectrum disorder (ASD) in the offspring. This study

examined the association between prenatal antibiotic exposure and ASD when the children were between 8–14 years of age. The study found that there was an approximately 40–60% increased risk of ASD with use of sulfonamides any time during pregnancy and penicillin during the second or third trimesters (Atladóttir, et al., 2012). A population-based cohort study in Canada that began in April 1998 did a similar analysis looking at the effect of any antibiotic exposure prenatally on the risk of ASD in children of at least 18 months of age. The results from this analysis showed an increased risk of ASD (HR 1.11) for children who had prenatal exposure to antibiotics (Hamad, et al., 2019).

There is also additional research that supports effects on the gut-brain-axis resulting in neurodevelopmental changes when looking at prenatal infections. A recent study examined the effects of bacterial infections during pregnancy on cognitive performance in early childhood. This study found that bacterial infections during pregnancy, especially later during pregnancy, were associated with lesser IQ scores in children by the age of 7 years old (Lee, et al., 2020). A meta-analysis of 10 studies was also conducted to assess the association of maternal chorioamnionitis (MC), a common infection of pregnancy, and neonatal motor development in preterm infants. This analysis found the pre-term infants of mothers who had MC had poorer mental and motor developments than those who had not been exposed to MC (Xiao, et al., 2018).

The limitations of the prior research pose a challenge in determining whether there is an association between prenatal exposure antibiotics and long-term effects on neurodevelopment. In addition, these studies examined an exposure of prenatal

antibiotics use or prenatal infections resulting in a lack of understanding whether there was an interaction that exists between the two exposures. The prior research is also limited in making broad characterizations of the association between maternal antibiotics use and subsequent child neurodevelopment due to restrictions in exposure and outcome variables such as exposures of MC versus all infections or outcomes of ASD rather than broad neurodevelopment categories. Due to the cost of lengthy follow ups and likelihood of participant attrition, most studies conducted on in utero exposures were limited to outcomes that were detectible during infancy. For outcomes that can take years to develop, such as more complex behavior or other developmental measures, early life outcomes that occur during infancy will not suffice.

The current study aims to increase the understanding of prenatal antibiotic use and long-term neurodevelopment outcomes of the offspring to fill these gaps in current research. Additional research in this area is important to provide more informed prescribing guidance to pregnant women on possible long-term side effects for their children exposed in utero. At present, older antibiotics are grandfathered in as safe due to limited short-term outcomes and new antibiotics are not tested on pregnant women due to potential ethical concerns. Observational research on long-term effects on the fetus is required to add to the body of literature to support the safety of this at-risk population.

## Study Design

This study is examining the cognitive and behavioral outcomes of children who previously participated in a multicenter, case-control study of risk factors for hemifacial microsomia (HFM). This analysis will be restricted to the controls of this study

population, or children without malformations. The study received approval from institutional review board at Boston University and was completed in compliance with the Health Insurance and Accountability Portability Act (HIPAA) standards.

## Study subjects

The identification of controls (child-mother pairs) has been previously published (Werler, et al., 2004; Collett, et al., 2011). In short, controls were identified through community pediatrician's in the vicinity of the 26 enrolling craniofacial centers across the United States and Canada. To be eligible, the controls were less than 4 years old, not adopted, and had no major malformations. Participants completed a standardized interview that collected data on demographics, reproductive history, and pregnancy exposures, including medications. Mothers of children were subsequently approached when the child was between 5 and 11 years of age to assess childhood neurodevelopment. Of the 826 controls that were eligible, 570 completed at least one of the childhood neurodevelopmental assessments.

## Study Measurements

### Antibiotic Use

Antibiotic use during early pregnancy (started within five months of her last menstrual period) is the primary exposure of interest. Illnesses and medication use were self-reported by the mother during the initial interview that took place within an average of 47 months of giving birth. The mother was prompted with a calendar of a six-month time period that began 1 month prior to her last menstrual period and ended 5 months after her last menstrual period based on the date of birth provided in order to focus

recollection to the timeframe of interest.

Medication use was obtained in two ways. First, medication use was captured indirectly by asking participants about illnesses that occurred during the six-month time period and then would subsequently be asked about medications to treat any reported illness. In this interview, participants were specifically asked about upper respiratory infection (URI) and sinusitis/sinus headache which were most pertinent for subsequent antibiotic use. Medication use was also directly ascertained through a prompted review of a medication list divided into 11 major drug categories, antibiotics being one of these categories. If the mother confirmed use, they would be requested to gather the bottle or prescription, if available, to provide further details on the medication. In any case of reported medication use, the mother was asked to provide additional information including diagnosis, start and stop date, frequency and dosage. Medications included in the medications list were classified based on active ingredient or class using the Boston University Slone Drug Dictionary, a computerized coding system that includes codes for prescription and non-prescription drugs, dietary supplements, and other natural products (Boston University Slone Epidemiology Center Slone Drug Dictionary).

For the purpose of this analysis, the exposed group is children of mothers with any reported antibiotic use during early pregnancy. The reference group for all analyses will be children of mothers who did not report antibiotic use during early pregnancy.

**Neurodevelopmental Outcomes**

The neurodevelopmental outcomes of the offspring were measured by four different assessments. These assessments included the Peabody Picture Vocabulary Test-

Third Edition (PPVT-III), the Beery-Buktenica Development Test of Visual Motor Integration-Fifth Edition (VMI-5), the Child Behavior Checklist (CBCL) and Teacher Report Form (TRF). The PPVT-III and VMI-5 focused on cognitive-based outcomes while the CBCL and TRF focused on behavior-based outcomes.

The PPVT-III is a norm-referenced measure of receptive vocabulary. Respondents were presented with increasingly difficult vocabulary words and shown four target pictures. They were asked to point to the picture that represents the word. Reliability is excellent, and convergent validity is supported by strong correlations with other measures of verbal ability and prediction of academic achievement (Dunn & Dunn, 1997). Higher scores on this test indicate better receptive vocabulary in the child.

Perceptual-motor skills were measured by the Beery-Buktenica Test of Visual Motor Integration-Fifth Edition (VMI-5). The VMI-5 is a norm-referenced measure of perceptual motor abilities. Respondents copy a series of 24 increasingly difficult geometric designs which were scored for accuracy by an examiner. The VMI-5 has good reliability, including strong test-retest stability and inter-scorer reliability among diverse examiners (e.g., ranging from psychologists to teachers). Validity of the VMI-5 is supported by convergence with other measures of visual perception and low correlations with verbal measures (Beery, Buktenica, & Beery, 2004). Higher scores indicate better perceptual motor abilities.

Mothers and teachers completed the Child Behavior Checklist (CBCL) and Teacher Report Form (TRF), respectively. These instruments were widely used in psychiatric research and with similar item content between tests, it allows for a

systematic comparison of child behavior. Both measures provide summary composite scales of internalizing behavior problems, externalizing behavior problems and total behavior problems. For these analyses, the total behavior problem scale was used across the two measures. T-scores were calculated for the total behavior problem scale on each test with a mean of 50 and an SD of 10. Both measures have well-established reliability and validity (Achenbach & Rescorla, 2001). Higher scores indicate greater behavioral problems.

**Statistical Analyses**

Demographic variables were summarized using frequencies and appropriate summary statistics. The summary statistics were presented based on exposure groups (antibiotic use or no antibiotic use). Demographic variables include maternal race, age, education, household income, marital status, region of the country, pre-pregnancy BMI, smoking status during pregnancy, and drinking status during pregnancy.

Antibiotic use was summarized by individual antibiotic and class of antibiotics. Due to limited samples size in antibiotic categorizations, the antibiotic categorizations will be presented as descriptive statistics only and will not be further analyzed for associations with the outcome.

Following identification of those exposed to antibiotics, diagnoses relating to their exposures were reviewed and categized by type, upper respiratory infection (URI), urogenital infection (UGI), or indications that were not typical of antibiotic use. Indications that were included with the URI categorization include pharyngitis streptococcal, otitis not otherwise specified (NOS), earache, common cold, acute

sinusitis, acute bronchitis, asthma, upper respiratory symptoms, and cough. Indications that were included with the UGI categorization include beta strep infection, kidney infection, cystitis, urinary tract infection, vaginitis, genital infection NOS, and vaginal bleeding. Indications that were included in the not applicable grouping included pain NOS and acne. Since this grouping of indications were inherently outliers, they were dropped from all analyses. The categorizations of URI and UGI were also combined to look at effects of any infection. Once these categorizations were determined using the above indications in the exposed group, they were then applied to the unexposed group

Average mean scores on the cognitive tests, PPVT-III and VMI-5, and the average total behavior problems T-score on the behavior assessment tools, CBCL and TRF, were calculated for children exposed and unexposed to antibiotics prenatally.

An adjusted linear regression model was used to calculate adjusted mean difference (MD) and 95% confidence interval (CI) for exposed versus unexposed using children unexposed to antibiotics prenatally as the reference. Potential confounders were identified theoretically and included maternal race, age, education, region of the country, pre-pregnancy BMI, smoking status during pregnancy, drinking status during pregnancy, URI, UGI and any infection.

In addition to the primary analysis, a secondary analysis was completed restricting the population to only those mothers who reported infection. Utilizing an adjusted linear regression model the analysis examined the effect of infections during pregnancy treated with antibiotics versus those left untreated on neurodevelopmental outcomes in the children of those mothers during early childhood. Analyses were performed using SAS

Software Version 9.3 and the GLM procedure (SAS Institute, Cary, NC).

## Results

### Cohort characteristics

Maternal characteristics of children exposed and unexposed to antibiotic use during early pregnancy are shown in Table 1. Of the 570 mother-child pairs included in this analysis, approximately 19% (n=111) reported antibiotic use in early pregnancy. Antibiotic users were more likely to be white/non-Hispanic mother (82% compared to 74.1% among the unexposed), obese (18.9% compared to 10.6%), and smokers (18.9% compared to 15.0%).

A total of 361 mothers reported indications relating to infections during early pregnancy across this population, with 103 women treating them with antibiotics. In the exposed group 78.4% reported indications relating to URI and 35.1% relating to UGI vs. 49.7% and 13.6%, respectively, in the unexposed group. There were 8 reports of antibiotics use with no accompanying infection-related diagnosis. The indications reported included cornea abrasion (n=2), infection prophylaxis (n=2), calculus kidney, pain not otherwise specified, bleeding gums and dental surgical operation not otherwise specified.

### Exposure characteristics

Antibiotic use was reported 131 times across the 111 subjects exposed. Fifteen mothers reported more than one incidence of antibiotic use during early pregnancy. The median length of use per incident was 7 days with a minimum length of 1 day and

maximum length of 280 days. Frequency of reported use by antibiotic class is described in Table 2.

**Primary Analysis**

The results of the adjusted multivariable regression analyses assessing the relationship between maternal antibiotic use during pregnancy and neurodevelopmental outcomes in the offspring are presented in Table 3. Antibiotic use during early pregnancy was associated with mixed associations across the neurodevelopmental measures. Within the cognitive outcomes, those children exposed to antibiotics in utero had PPVT-III scores 1.12 points better than those unexposed (adjMD 1.12; 95% CI -1.89, 4.13) indicating better receptive vocabulary in those exposed, but VMI-5 scores 0.48 points worse than those unexposed (adjMD -0.48; 95% CI -2.90, 1.93) indicating worse perceptual motor abilities in those exposed. Confidence intervals around these associations were wide indicating a lack of precision and included the null value. Within the behavioral outcomes, those exposed had TRF scores 2.60 points greater than those unexposed (adjMD 2.60, 95% CI 0.50,4.69) indicating worse behavioral problems as reported by the teacher in those exposed and CBCL scores 2.60 points worse than those unexposed (adjMD 2.60; 95% CI 0.44, 4.76) indicating worse behavioral problems as reported by the mother in those exposed. Both associations also had wide confidence intervals indicating a lack of precision, however, the intervals did not include the null value.

To address confounding by indication, the regression models were also adjusted by URI, UGI and any infection. When adjusting for URI, mean differences for PPVT-III

and VMI-5 scores were strengthened, however, remained directionally opposite and with wide confidence intervals including the null value. Those exposed had PPVT-III scores 1.37 better than those unexposed (adjMD 1.37, 95% CI -1.70, 4.43), but VMI-5 scores 0.77 points worse than those unexposed (MD 0.77; 95% CI -3.23, 1.67). In this model, the mean differences for TRF and CBCL were reduced compared to the demographic adjusted mean differences. Those exposed had TRF scores 2.06 worse (adjMD 2.06, 95% CI -0.08, 4.21) and CBCL scores 1.66 points worse (adjMD 1.66; 95% CI -0.54, 3.86) than those unexposed. When adjusting for UGI, the mean difference for PPVT-III was greatly reduced compared to both the demographic and URI adjusted models so while still showing better receptive vocabulary in those exposed, the magnitude was greatly reduced with those exposed having PPVT-III scores 0.72 points better than those unexposed (adjMD 0.72; 95% CI -2.39, 3.84). There was very little change in the mean differences for VMI-5 and CBCL from the demographic adjusted model. Those exposed had VMI-5 scores 0.50 points worse (adjMD -0.05, 95% CI -2.99, 2.00) and CBCL scores 2.58 points worse than those unexposed (adjMD 2.58, 95% CI 0.35, 4.81). After adjusting for UGI, the mean difference for TRF scores increased, showing a greater magnitude in the behavior problems as reported by the teacher in those exposed, compared to demographic and URI adjusted models. Those exposed had TRF scores 3.31 points worse than those unexposed (adjMD 3.31; 95% CI 1.16, 5.47). Lastly, after adjusting for any infection, the mean differences for PPVT-III, TRF, and CBCL scores were consistent with the demographic and URI adjusted models. The mean difference for VMI-5 scores, however, is increased showing worsened perceptual motor abilities in

those exposed compared to the demographic and URI adjusted models with those exposed having VMI-5 scores 1.06 points worse than those unexposed (adjMD -1.06, 95% CI -3.56, 1.44). Overall, there is a consistency through the crude, demographic, URI and UGI adjusted models that those exposed have better PPVT-III scores indicating better receptive vocabulary, but worse VMI-5, CBCL and TRF scores indicating worse perceptual motor abilities and behavioral problems as reported by the mother and teacher, respectively, than those unexposed.

**Secondary analyses**

The results of the adjusted multivariable regression analyses restricted to only those mothers reporting infections during pregnancy to assess the relationship between treated infections during pregnancy and neurodevelopmental outcomes in the offspring with untreated infections as the reference group are presented in Table 4

After restricting to only those mothers with any reported infection-related diagnosis during early pregnancy, the effects of antibiotic use on the neurodevelopmental outcomes of their offspring in early childhood remain similar to the unrestricted analyses adjusted by any infection presented in Table 3. Although results had wide confidence intervals indicating a lack of precision, children whose mothers had treated infections during early pregnancy had PPVT-III scores better than those unexposed, while having worse scores across VMI, TRF and CBCL assessments consistent with the unrestricted primary analyses. Those exposed had PPVT-III scores 1.90 points better (MD 1.90; 95% CI -1.48, 5.28), but VMI-5 scores 0.98 points worse (MD -0.98; 95% CI -3.64, 1.68), CBCL scores 2.05 points worse (MD 2.05, 95% -0.28, 4.39), and TRF scores 1.92 points

worse (MD 1.92, 95% CI -0.47, 4.31) than children whose mothers had infections that were not treated during early pregnancy.

Differences in neurodevelopmental scores in children whose mothers reported treated URI related diagnoses during early pregnancy were directionally consistent with the model for any infection, however, found greater differences in magnitude across all measures than those differences found in the unrestricted analyses adjusted by URI presented in Table 3. When restricting to treated URI related diagnoses during early pregnancy, children whose mothers were treated had PPVT-III scores 3.10 better (MD 3.10; 95% CI -0.61, 6.81), but VMI-5 scores 1.03 points worse (MD -1.03; 95% CI -3.96, 1.90), CBCL scores 2.01 points worse (MD 2.01, 95% -0.55, 4.58), and TRF scores 2.28 points worse (MD 2.28, 95% CI -0.30, 4.87) than children whose mothers were untreated.

Among those children who mothers had treated UGI related diagnoses during early pregnancy, there was an inconsistency in the associations found compared to those associations presented in the unrestricted analyses adjusted by UGI in Table 3. The mean difference for PPVT-III was directionally opposite from the rest of the models with children of mothers who reported a treated UGI related diagnosis during early pregnancy having a PPVT-III score 3.42 points worse than those children whose mothers were untreated (MD -3.42, 95% -8.77, 1.92) indicating worse receptive vocabulary in those children of treated mothers. The mean VMI-5 scores were also directionally opposite from the rest of the models with children of mothers who reported a treated UGI during early pregnancy having VMI-5 scores 1.22 points better than those children whose mother reported an untreated UGI during early pregnancy (MD 1.22; 95% CI -3.52,

5.97). While the mean differences for CBCL and TRF scores remain directionally consistent with the unrestricted UGI adjusted analysis, the mean difference for TRF scores is much less in magnitude comparatively. Those exposed had CBCL scores 3.45 points worse than those unexposed (adjMD 3.45; 95% CI -0.36, 7.26) and TRF scores 0.95 points worse than those unexposed (adjMD 0.95; 95% CI -3.63, 5.53).

Overall, results across the any infection and URI restricted analysis found consistent results and were also consistent with the unrestricted analysis, however, the UGI restricted model emerged with some inconsistent results. The any infection and URI restricted analysis found that those treated had children with better PPVT-III scores, but worse VMI-5, CBCL, and TRF scores. For the UGI restricted analysis, children of treated mothers had better VMI-5 scores, but worse PPVT-III, CBCL and TRF scores indicating better perceptual motor abilities, but worse receptive vocabulary and behavior problems as reported by the mother and teacher, respectively. Results from these analyses have wide confidence intervals indicating a lack of precision.

## Discussion

The results of these analyses showed a consistent trend that prenatal antibiotic exposure was associated with greater childhood behavioral problems as indicated by the increases in CBCL and TRF scores. Mean CBCL and TRF scores across exposed and unexposed groups remain below the borderline and clinical T-score cut-offs so while those exposed have higher scores they do not indicate clinical intervention (Achenbach & Rescorla, 2001). Associations of prenatal exposure to antibiotics and cognitive outcomes, PPVT-III and VMI-5, were less consistent. Children with prenatal antibiotic exposure

had higher PPVT-III scores indicating better receptive vocabulary, but lower VMI-5 scores indicating worse perceptual motor abilities than those unexposed.

After adjusting the primary analysis by overall infection, URI and UGI, trends maintained the same direction with some small differences in magnitude. Similar results were also seen in the secondary restricted analyses, however, in the UGI restricted model, both PPVT-III and VMI had directionally inconsistent results with the rest of the primary and secondary analyses. The incidence of UGI in this sample is much less compared to URI so the primary analysis results of URI and any infection were more similar than those of UGI. Due to the limited sample of UGIs, firm conclusions cannot be drawn from the UGI restricted model as the inconsistent results may be due to variability.

With the ability to follow up with the same mother-child pairs of the original study, we're able to examine in utero exposure with long term neurodevelopmental outcomes. Mothers were asked to recall use of antibiotics and illness after delivery which may have resulted in exposure misclassification, although the interview was standardized and the average time between delivery and the interview was 1 year. Additionally, the CBCL was completed via the mother's report and if the mother had difficulty with recall of antibiotics, it's possible that she also had a poor assessment of her child's behavior creating dependent misclassification. Since we also have the teacher's assessment of the child's behavior via the TRF we were able to obtain feedback on the outcome without influence or knowledge of the exposure and results were similar.

Since antibiotic use and neurodevelopmental outcomes were not the primary

purpose of the original study design, there were limitations in the collection of these data. Antibiotics, and more generally medications, were collected up until six months after the mother's last menstrual period. Therefore, most of these data were restricted to the first two trimesters of pregnancy reducing any conclusions drawn to be restricted to early pregnancy. Diagnoses based on medication use were also prompted responses within the interview with URI being one of these categories. This provided the opportunity to an overreporting of URI and potential underreporting of other indications that were not prompted responses, like UGI, which causes limitations in drawing conclusions across these groups. Also due to the prompted responses, antibiotic use was closely linked with occurrence of infection, especially URI, making it difficult to distinguish associations with the outcomes due to antibiotics versus infections. The sample size is also limited which increases variability ultimately making it difficult to show statistical significance and draw strong conclusions but will add to the body of literature to help identify potential associations. Additionally, since we were limited in the number exposed, we were unable to do further associations by grouping the antibiotics by class or FDA risk categorization which may have better results given all antibiotics do not interact with the body in the same manner. Overall, this study, even with its limitations, should help bring to light potential safety risks in using antibiotics during pregnancy and support the risk benefit analysis required when prescribing these medications to pregnant women.

# Tables

## Table 1

Maternal characteristics during pregnancy of children exposed and unexposed

| Characteristic | Exposed *(n=111)* | | Unexposed *(n=459)* | |
|---|---|---|---|---|
| | **n** | **%** | **n** | **%** |
| **Maternal Race** | | | | |
| White/Non-Hispanic | 91 | 82.0% | 340 | 74.1% |
| Hispanic | 8 | 7.2% | 59 | 12.9% |
| Black | 10 | 9.0% | 39 | 8.5% |
| Other | 2 | 1.8% | 21 | 4.6% |
| **Maternal Age** | | | | |
| <25 years | 16 | 14.4% | 99 | 21.6% |
| 25–34 years | 76 | 68.5% | 282 | 61.4% |
| ≥ 35 years | 19 | 17.1% | 78 | 17.0% |
| **Maternal Education** | | | | |
| <12 years | 30 | 27.0% | 142 | 30.9% |
| 12-15 years | 30 | 27.0% | 106 | 23.1% |
| ≥ 15 years | 51 | 46.0% | 211 | 46.0% |
| **Family Income** | | | | |
| ≤$15,000 | 9 | 8.1% | 38 | 8.3% |
| $15,001-$25,000 | 9 | 8.1% | 36 | 7.9% |
| $25,001-$35,000 | 7 | 6.3% | 61 | 13.3% |
| $35,001-$65,000 | 41 | 36.9% | 138 | 30.1% |
| >$65,000 | 41 | 36.9% | 160 | 34.9% |
| Missing | 4 | 3.6% | 25 | 5.5% |
| **Marital Status** | | | | |
| Married | 85 | 76.6% | 361 | 78.7% |
| Other | 26 | 23.4% | 98 | 21.4% |
| **Region** | | | | |
| Northeast | 16 | 14.6% | 78 | 17.0% |
| Mid-Atlantic | 13 | 11.8% | 50 | 10.9% |
| Midwest | 36 | 32.7% | 131 | 28.6% |
| South | 22 | 20.0% | 68 | 14.9% |
| West | 23 | 20.9% | 131 | 28.6% |
| **Pre-pregnancy BMI** | | | | |
| <18.5 | 6 | 5.4% | 13 | 2.9% |
| 18.5-24.9 | 66 | 59.5% | 293 | 64.7% |
| 25-29.9 | 18 | 16.2% | 99 | 21.9% |
| 30+ | 21 | 18.9% | 48 | 10.6% |

| Characteristic | Exposed *(n=111)* | | Unexposed *(n=459)* | |
|---|---|---|---|---|
| | **n** | **%** | **n** | **%** |
| **Smoking Status** | | | | |
| No | 90 | 81.1% | 390 | 85.0% |
| Yes | 21 | 18.9% | 69 | 15.0% |
| **Drinking Status** | | | | |
| No | 102 | 91.9% | 412 | 89.8% |
| Yes | 9 | 8.1% | 47 | 10.2% |
| **Any Infection** | | | | |
| No | 8 | 7.2% | 201 | 43.8% |
| Yes | 103 | 92.8% | 258 | 56.2% |
| **Upper Respiratory Infection (URI)** | | | | |
| No | 24 | 21.6% | 230 | 50.3% |
| Yes | 87 | 78.4% | 227 | 49.7% |
| **Urogenital Infection (UGI)** | | | | |
| No | 72 | 64.9% | 395 | 86.4% |
| Yes | 39 | 35.1% | 62 | 13.6% |

**Table 2**

Frequency of antibiotics used by class

| Antibiotic Class | Frequency | |
|---|---|---|
| | n | % |
| Ampicillin | 42 | 32.1% |
| Sulfonamide | 3 | 2.3% |
| Tetracycline | 3 | 2.3% |
| Cephalosporin | 18 | 13.7% |
| Macrolides | 20 | 15.3% |
| Penicillin | 6 | 4.6% |
| Other antibiotics | 40 | 30.5% |

**Table 3**

Mean difference in neurodevelopmental test scores of children among mothers who used antibiotics compared to those that did not antibiotics during early pregnancy

| Test | N | | Mean (SD) | | Mean Difference (95% CI) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Antibiotic Use | No Antibiotic Use | Antibiotic Use | No Antibiotic Use | Crude | Adjusted | Adjusted for Any Infection | Adjusted for URI | Adjusted for UGI |
| **PPVT-III** | 92 | 377 | 107.75 (13.60) | 105.92 (14.39) | 1.83 (-1.43, 5.08) | 1.12 (-1.89, 4.13) | 1.56 (-1.59, 4.70) | 1.37 (-1.70, 4.43) | 0.72 (-2.39, 3.84) |
| **VMI-5** | 92 | 376 | 96.22 (10.43) | 96.83 (10.77) | -0.61 (-3.01,1.79) | -0.48 (-2.90, 1.93) | -1.06 (-3.56, 1.44) | -0.77 (-3.23, 1.67) | -0.50 (-2.99, 2.00) |
| **TRF** | 96 | 405 | 50.51 (9.99) | 47.76 (9.38) | 2.69 (0.59, 4.80) [ɪ] | 2.60 (0.50, 4.69) [ɪ] | 2.01 (-0.19, 4.20) | 2.06 (-0.08, 4.21) | 3.31 (1.16, 5.47) [ɪ] |
| **CBCL** | 109 | 457 | 49.32 (10.16) | 46.75 (10.46) | 2.53 (0.37, 4.70) [ɪ] | 2.60 (0.44, 4.76) [ɪ] | 1.80 (-0.47, 4.06) | 1.66 (-0.54, 3.86) | 2.58 (0.35, 4.81) [ɪ] |

*Note*: *Adjusted mean differences were adjusted by maternal age, race, education, region, pre-pregnancy BMI, smoking status, drinking status;* [ɪ] *$p<0.05$*

**Table 4**

Neurodevelopmental test scores among children whose mothers reported treated infections during early pregnancy compared to those children whose mother reported untreated infection during early pregnancy

| **Test** | **Any Infection** | | | **URI** | | | **UGI** | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | Mean (SD) | Mean Difference (95% CI) | N | Mean (SD) | Mean Difference (95% CI) | N | Mean (SD) | Mean Difference (95% CI) |
| **PPVT-III** | 303 | 106.65 (14.39) | 1.90 (-1.48, 5.28) | 266 | 106.64 (14.44) | 3.10 (-0.61, 6.81) | 22 | 109.77 (12.40) | -3.42 (-8.77, 1.92) |
| **VMI-5** | 305 | 96.97 (10.89) | -0.98 (-3.64, 1.68) | 267 | 96.89 (11.03) | -1.03 (-3.96, 1.90) | 23 | 98.26 (11.29) | 1.22 (-3.52, 5.97) |
| **TRF** | 319 | 49.24 (9.53) | 2.05 (-0.28, 4.39) | 279 | 49.58 (9.64) | 2.01 (-0.55, 4.58) | 26 | 45.62 (9.19) | 3.45 (-0.36, 7.26) |
| **CBCL** | 360 | 48.26 (10.48) | 1.92 (-0.47, 4.31) | 313 | 48.92 (10.35) | 2.28 (-0.30, 4.87) | 31 | 43.55 (11.79) | 0.95 (-3.63, 5.53) |

*Note: Mean differences were adjusted by maternal age, race, education, region, pre-pregnancy BMI, smoking status, and drinking status*

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## Curriculum Vitae